Technical Bulletin

Oxaloacetate Assay Kit

Catalogue number MAK515

Product Description

Oxaloacetate (OAA) is an intermediate in the citric acid cycle and participates in gluconeogenesis. OAA is formed by the oxidation of malate, by deamidation of aspartate or by condensation of CO_2 with pyruvate or phosphoenolpyruvate.

The Oxaloacetate Assay Kit provides a simple, direct and automation-ready procedure for measuring oxaloacetate concentration. OAA is converted into pyruvate which is then oxidized with the conversion of the dye into a colored and fluorescent form. The color intensity of the oxidized dye at 570 nm or fluorescence intensity at λ_{ex} = 530 nm/ λ_{em} = 585 nm is directly proportional to the oxaloacetate concentration in the sample.

The linear detection range of the kit 7 to 400 µM oxaloacetate for the colorimetric assays and 1 to 40 µM for the fluorometric assays. The kit is suitable for oxaloacetate determination in plasma, serum, tissue and culture media.

Components

The kit is sufficient for 100 colorimetric OR Fluorometric assays in 96-well plates.

- Developer 10 mL
  Catalogue Number MAK515A
- ODC Enzyme 120 µL
  Catalogue Number MAK515B
- Dye Reagent 120 µL
  Catalogue Number MAK515C
- Oxaloacetate Standard 1 vial
  Catalogue Number MAK515D

Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- microcentrifuge tubes
- Dounce tissue grinder set (Catalogue Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of ≥ 14,000 rpm.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Colorimetric Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Tissue and Cell Samples

1. Homogenize the tissue and cell Samples (2 x 10^6) in 100 µL PBS.
2. Centrifuge at 14,000 rpm for 5 minutes.
3. Deproteinate the supernatant using a 10 kDa spin filter (Catalogue UFC8010 or equivalent).

Serum and Plasma Samples

Samples must be deproteinated and an internal Standard should be used.

Cell Culture Media

Avoid media with high pyruvate concentrations when assaying Cell Culture Media Samples.

(For example, DMEM, L-15, F12, etc.)

Note: If using an internal Standard, Samples will need three separate reactions.

Sample Blank

1. Transfer 20 µL of each Sample to separate wells.
2. To each Sample Blank well, add 5 µL of purified water.

Internal Standard

(Required for serum and plasma Samples)

1. Transfer 20 µL of each Sample to separate wells.
2. Prepare 500 µL 80 µM OAA Standard by mixing 100 µL 400 µM OAA standard and 400 µL purified water.
3. For colorimetric and fluorometric assays, to each Internal Standard well, add 5 µL of 80 µM OAA Standard.

Add 20 µL of each Sample and 5µl of dH_2O to two separate wells in a 96 well plate.

Standard Curve Preparation

1. Prepare 10 mM stock by dissolving it with 100 µL. purified water.
2. Prepare a 400 µM OAA Standard by diluting 20 µL of the 10 mM Standard with 480 µL purified water.
3. Further, dilute Standards in 1.5mL centrifuge tubes as mentioned in Table 1.

Note:

1. Keep standard cold and store at -20 °C.
2. Reconstituted OAA Standard should be used within 2 weeks.
3. If assaying culture media with phenol red, dilute the Oxaloacetate Standard in culture media.

Table 1.
Dilution of Standard

No.	400 µM OAA Standard	Purified H_2O	Oxaloacetate (µM)
1	100 µL	0 µL	400
2	60 µL	40 µL	240
3	30 µL	70 µL	120
4	0 µL	100 µL	0

4. Transfer 20 µL Standards into separate wells of a clear flat-bottom 96-well plate.

Working Reagent Preparation

1. Mix enough reagents for the number of assays to be performed. For each Standard and Sample well, prepare the Working Reagent according to Table 2.

Table 2.
Preparation of Working Reagents

Reagent	Working Reagent	Blank Working Reagent
Developer	85 µL	85 µL
ODC Enzyme	1 µL	-
Dye Reagent	1 µL	1 µL

2. Transfer 80 µL Working Reagent into each reaction well. Add 80 µL Blank Working Reagent to the Sample Blank Wells. Tap plate to mix.

Measurement

1. Incubate the plate, protected from light, for 15 minutes at room temperature.
2. Read the optical density at 570 nm.

Fluorometric Procedure

1. Dilute the Standards prepared in Colorimetric Procedure 1:10 in purified water. If an internal Standard is used, use the same concentration as described in the Colorimetric Procedure (for example, 5 µL of 80 µM OAA).
2. Transfer 20 µL of the Standards and 20 µL of Sample (2 wells per Sample if a Standard curve is used; 3 wells per sample if an internal Standard is used, see Colorimetric Procedure) into separate wells of a black 96-well plate.
3. Add 80 µL of appropriate Working Reagent (see Colorimetric Procedure) to each well. Tap the wellplate to mix.

Measurement

1. Incubate for 15 minutes at room temperature.
2. Read fluorescence at λ_{ex} = 530 nm and λ_{em} = 585 nm.

Results

1. Calculate ΔOD or ΔF by subtracting the reading (OD or fluorescence intensity F) of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD or ΔF against Standard concentrations and determine the slope of the Standard curve.
3. Calculate the Oxaloacetate Concentration of samples using the below given equation:

$$\text{Oxaloacetate (µM)} = \frac{R_{Sample}-R_{Blank}}{\text{Slope}(\mu M^{-1})} \times DF$$

If an internal standard was used, the sample Oxaloacetate concentration is computed as follows:

$$\text{Oxaloacetate (µM)} = \frac{R_{Sample}-R_{Blank}}{R_{Standard}-R_{Sample}} \times \frac{80}{4} \times DF$$

Where:

R_{Sample} = OD or fluorescence intensity (F) reading of Sample

R_{Blank} = OD or fluorescence intensity (F) reading of Blank

80 = Concentration in µM of Internal Standard

4 = The volume of the Internal Standard is 4× lower than the Sample volume. In order to correct the calculation, the Internal Standard concentration is divided by 4.

DF = Sample dilution factor (DF = 1 for undiluted samples

Conversions: µM oxaloacetate equals 13.1 mg/L, 0.00131% or 13.1 ppm.

Figure 1.

Typical Colorimetric Oxaloacetate Standard Curve.

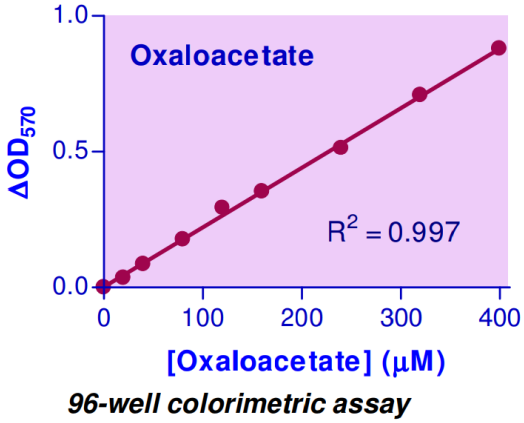
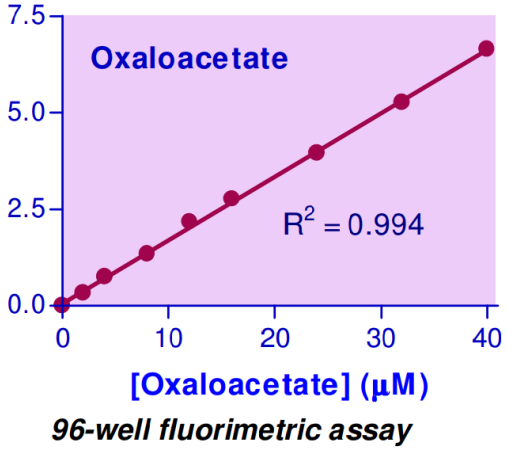


Figure 2.

Typical Fluorometric Oxaloacetate Standard Curve



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